

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: )  
Hea Young Park Choo et al. )  
Serial No.: 10/789,725 ) Group Art Unit:1614  
Filed: February 27, 2004 ) Examiner: SPIVACK, PHYLLIS G  
)

For: METHOD FOR INHIBITING 5-LIPOXYGENASE USING A BENZOXAZOLE DERIVATIVE OR AN ANALOGUE THEREOF

Commissioner for Patents  
P. O. Box 1450  
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. §1.132

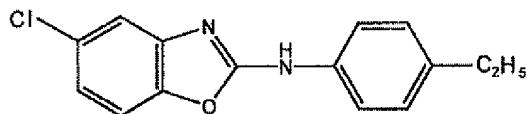
Sir:

I, Hea Young Park Choo, being a citizen of the Republic of Korea and presently residing at Hyundai Apt. 86-603, Apgujung-dong, Kangnam-gu, Seoul 135-905, Republic of Korea, do declare:

That I am one of the co-inventors of the invention disclosed in the above-identified application, and hence am fully familiar with the subject matter therein; and

That I have conducted the following experiment, in order to demonstrate that the compound according to the subject application, which inhibits 5-lipoxygenase is clinically effective in preventing or treating leukotriene-related diseases. The compound of Example 9 having the structure shown below, which has 5-

lipoxygenase inhibiting activity of  $IC_{50}=0.95\mu M$ , was used as a test compound in the following experiment.



**<In vivo evaluation of asthma treatment effect by 5-lipoxygenase inhibition >**

The *in vivo* efficacy of the test compound for treating asthma was evaluated together with Zileuton (Zyflo<sup>TM</sup>) (Abbott Laboratories), a comparative compound which is a known 5-lipoxygenase inhibitor, as follows.

**(1) Mice modeling for sensitization and airway challenge**

8 Week old female BALB/c mice were obtained from Korean Research Institute of Chemistry Technology, kept in a laminar flow cabinet and divided into 3 groups (5-6 mice per group). The 3 groups of mice were respectively subjected to the following treatments: (1) sham-sensitization plus challenge with phosphate-buffered saline (PBS; ipNeb); (2) sensitization plus challenge with ovalbumin (OVA) (Sigma A5503; Sigma, St. Louis, MO) (ipNeb); and (3) sensitization with OVA (ip) plus challenge with OVA (Neb) and a drug (the test compound or Zileuton) (po).

Specifically, the test mice were sensitized with intraperitoneal injection of 20  $\mu$ g OVA with 4 mg of adjuvant aluminum hydroxide on days 0 and 11. The mice were challenged through the airways with OVA (1% in PBS) on day 11, 21, 22, 23 and 25 after the initial sensitization to induce inflammation. A 50mg/kg bodyweight dosage of a test drug was orally administered once a day on days 21-25. The mice were assessed 24 hours after the last challenge for the suppressive effect of the drug on the airways of allergic asthma.

**(2) Determination of Airway Hyperresponsiveness**

Airway hyperresponsiveness (AHR) was determined 24 hours after the final challenge. Each mouse was placed in a barometric plethysmographic chamber

and challenged with aerosolized PBS for 3 min, followed by challenging with aerosolized methacholine whose concentration was changed from 0 to 30 mg/ml, at intervals of 5 mg/ml, each for 3 min, and AHR was recorded for 5 minutes thereafter. To evaluate the degree of AHR, the enhanced pause Penh values of the drug sample was obtained during each methacholine challenge, and expressed as a percentage value of the basal Penh value obtained in control (PBS) challenge, in which the Penh value was calculated as follows:

$$\text{Penh} = [\text{T}_e /(\text{RT}-1)] \times [\text{PEF} / \text{PIF}]$$

wherein,  $\text{T}_e$  is expiratory time; RT is relaxation time; PEF is peak expiratory flow; and PIF is peak inspiratory flow.

The results are shown in Table I.

Table I

Methacholine Concentration	0mg/ml	5mg/ml	10mg/ml	20mg/ml	30mg/ml
Control (OVA only)	0.656	1.376	2.418	2.878	3.277
Zileuton	0.567	1.673	2.344	2.588	2.866
Compound of Ex. 9	0.516	0.919	1.515	1.876	2.406

As shown in Table I, the inventive compound of Example 9 exhibited significantly low hyperresponsiveness.

### (3) Measurement of IL-4, IL-5 and IL-13 level

The mice were sacrificed by pentobarbital overdose (Sigma P3761) 24 hours after AHR measurement and tracheotomy was performed. After ice-cold PBS (0.5 ml) was introduced into the lung, bronchoalveolar lavage fluid (BALF) was obtained by repeating aspiration three times (total 1.5 ml) via tracheal cannulation. BALF was centrifuged at 4 °C, and the supernatant was collected and stored at -70 °C until use. The amounts of cytokines IL-4, IL-5 and IL-13 in BALF were measured with a specific mouse ELISA kit (R&D Systems; Minneapolis, MN) and the results are

shown in Tables II to IV.

Table II

IL-4 Amount (ng/ml) in BALF							
	1	2	3	4	5	a.v. <sup>1)</sup>	s.d. <sup>2)</sup>
Control (OVA only)	422.03	476.73	316.4	424.01	470.2	421.872	64.1715
Zileuton	274.43	321.74	358.3	266.8	204.9	285.234	58.2696
Compound of Ex. 9	212.46	112.94	130.24	218.2	143.6	163.486	48.6007

1) Average value  
2) Standard deviation

Table III

IL-5 Amount (ng/ml) in BALF							
	1	2	3	4	5	a.v. <sup>1)</sup>	s.d. <sup>2)</sup>
Control (OVA only)	248.82	227.21	219.52	215.05	323	246.717	44.5694
Zileuton	113.31	161.01	181.44	219.27	214.3	177.864	43.3198
Compound of Ex. 9	85.972	118.27	152.68	113.8	83.83	110.911	28.1174

1) Average value  
2) Standard deviation

Table IV

IL-13 Amount (ng/ml) in BALF							
	1	2	3	4	5	a.v. <sup>1)</sup>	s.d. <sup>2)</sup>
Control (OVA only)	128.45	123.16	105.09	191.87	165.9	142.89	35.1914
Zileuton	124.37	101.3	83.84	85.45	104.3	99.842	16.4802
Compound of Ex. 9	83.73	78.437	55.266	58.76	83.37	71.913	13.8166

1) Average value

## 2) Standard deviation

As shown in Tables II to IV, the inventive compound of Example 9 exhibited improved activity for inhibiting the formation of cytokines IL-4, IL-5 and IL-13 in BALF.

### (4) Histopathology studies

The lung tissue from each sacrificed mouse was fixed in 10% neutral-buffered formalin for 20 to 24 hours, embedded in paraffin, sliced into 4  $\mu\text{m}$  thickness sections, and stained with H-E solution (hematoxylin; Sigma MHS-16 and eosin, Sigma HT110-1-32). Subsequently, the stained tissue was mounted and cover-slipped with Dako-mounting medium (Dakocytomation; Denmark Carpinteria CA). The degree of inflammatory cell infiltration in lung sections, specifically the degree of peri-bronchiole and peri-vascular inflammation was evaluated using a specific standard scale, i.e., scoring with 0–3 (0, no inflammatory cell; 1, few inflammatory populations; 2, a thin ring of inflammatory cells (one to five cell-layer deep); 3, a thick ring of inflammatory cells (more than five cell-layer deep) and averaged. The results are shown in Table V.

Table V

Inflammation Score	Control (OVA only)	Zileuton	Compound of Ex. 9
Average value	2.225	1.835	1.501
Standard deviation	0.224	0.195	0.154

As shown in Table V, the inventive compound of Example 9 has good suppressive effect on the leukocyte infiltration.

It is well known in the art that leukotriene-related diseases have been associated with the activity of 5-lipoxygenase as one of various etiologies, and thus, the disease induced by the activity of 5-lipoxygenase among various etiologies can be treated by inhibiting 5-lipoxygenase.

Accordingly, I believe that the inventive compound having good inhibiting activity of 5-lipoxygenase is also effective in preventing or treating leukotriene-related diseases such as pertussis, psoriasis, rheumatic arthritis, arthritis, inflammatory bowel disease, cystic fibrosis, acute/chronic bronchitis, cardiac myoischemia,

cardiac anaphylaxis, ischemia and allergic rhinitis, as well as asthma.

I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and, further, that these statements made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patents issuing thereon.

Further the deponent saith not.

Date: 9<sup>th</sup> day of March, 2007

Hea Young Choo

(Hea Young Park Choo)